Use of Ozone to Treat Wounds

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Abstract. Ozone has a high potential to kill pathogenic factors. Ozone therapy improves pathologic conditions and may treat infectious wounds such as bedsores and infections due to burning and specially the wounds due to confine to bed in hospital for a long time. Recent studies indicated that chronic wound is increasing in communities; many people suffer from it every year and it has many economic and social problems for the communities. Ageing process of populations and diseases related to ageing such as diabetes, arterial diseases and trauma are the essential causes. Ozone role in treating chronic wounds has been proposed since many years ago and some studies have been done in this regard. Ozone is 9 atom oxygen which is colourless and has loud smell; it was used for the first as medicament in First World War; it was used to treat the German soldiers' gaseous gangrene; ozone has been become more valuable to treat diseases since that time. This material is mixed with oxygen with low concentrations in medicine. Studies indicated when the material is used with more local oxygen it plays some role in stimulating growth factors production, controlling inflammatory processes and vascular enlargement to treat some diseases. The mechanisms involved in ozone function in living tissues may play some role in accelerating the healing of chronic wounds. Considering chronic wounds treatment is long and hard it is very important to find new treatment mechanisms.

Keywords: Ozone, ozone therapy, types of wounds

Introduction

Wound includes the rupture of epithelium or any covering in any part of body such as skin, mucosa of digestive system, etc. so the treatment is different for each place (1). The wounds may be in different parts of body such as internal organs like digestive system or outer ones such as skin, but generally wounds are divided in two types: acute and chronic (2). It had better examine a wound healing process in order to define acute and chronic wounds; each wound has four steps: healing process begin in first seconds of the wound formation in a way that inflammatory cells go towards the center of the wound namely they attack there; this is done to remove the bacteria and pollutions there and usually it lasts four days and then it is the turn of secretion of inflammatory intermediates. In fact, wound healing is both harmonic and physiologic namely both body physiology and hormones play some role in the healing (3, 4); the second step usually lasts from the fourth to tenth or twelfth day during which the inflammatory cells enter into the wound hole and begins to multiply in order to create a scaffold and fill the wound with it to heal the wound. The third step of the wound healing is from tenth to twelfth day during which the cells' attack and multiplication have special discipline in a way that the body removes the wastes from the wound and the wound is forming like surrounding tissues which is known as 'Modeling' or 'Wound Change' (5).

Each wound has inflammation at first and this inflammation decreases in third and fourth days and becomes somehow prominent and then changes its model in 6 – 12 months to approach the neighbor tissue. The last step includes the removal of additional parts known as 'Wound Scar' or 'Mending Place'. Wound Scar disappear gradually and becomes a normal tissue. Of course, wound scar may continue genetically, does not disappear or disappear completely (6). Chronic or deep wounds appearance
encounter the patient with different disorders and even may lead to mutilation in some acute cases. Fortunately considerable medical developments and new useful methods have decreased considerably pains due to such wounds (7).

Chronic wound is a wound which has not improved and has not returned to normal condition of a sound tissue after three months (1,2). Each wound has potential to become chronic. Diabetes, arterial insufficiencies and chronic pressure on a place due to confining to bed are frequent causes of chronic wounds. Diabetes and vascular disorders lead to skin rupture and chronic wounds specially in the older patients and considering the populations are ageing more in actual communities it plays an important role in chronic wounds increase; due to the features of chronic wounds and necessity of continuous treatments it is necessary to allocate many economic and spiritual sources to public health throughout the world (2,3). Odourless and unstable O3 includes three atoms and is unstable due to its mesomeric conditions. This gas has a loud odour and was discovered in 19th century. German physicians used it in medical treatments for the first time in First World War to treat German soldiers' gaseous gangrene (7).

However, ozone was known as a gas to protect the Earth against UV in the stratosphere layer of the atmosphere and was considered harmful and poisonous in troposphere layer until recent years. No attention was paid to the treatment roles of these molecules, but the remedial effects of these molecules mixed with O2 have been examined in recent years (8). In recent years the function of the ozone effect in treatment process has been defined and the effective dose has been defined exactly; hence, its poisonous risk has been minimized as much as possible. Besides, different techniques to use it have been developed considerably (9,10).

Ozone use in medicine is presented in very diluted ozone – oxygen mixtures in 0.05 – 5 percent concentrations equal about 1 – 110 microgram per milliliter; if ozone is used in mentioned concentrations and the safety points and related directions are observed, it has no risk in remedial view (11). In this study first we describe ozone mechanisms' function and different methods of ozone therapy; then we examine briefly the frequent causes and treatments of chronic wounds and the ozone function and effect in the wounds treatment.

Ozone effects on body:

Ozone increases white cells and these cells protect body against viruses, bacteria, fungi and cancer and considering white cells may not function when there is not oxygen they are not able to destroy the attackers and even cannot defend sound and normal cells (Allergic reactions). It is noteworthy that ozone use for a long time increases oxygen in blood and then the allergies decrease, too (12).

2 – When there is oxygen the interferon increases considerably. The interferons are global proteins organizing all parts of the body immunity system; some of them are created by the cells contaminated by viruses. These global proteins warn the sound cells near each other about contamination probability in a way that they themselves are presented as separated cells so viral multiplication is prevented. Interferons are created by white cells, connective tissue and muscles. The interferon rate may sometimes increase 400 – 900 percent by ozone. It is noteworthy that the interferons play an important role in controlling phagocytic cells; these cells swallow abnormal cells and destroy them. Artificial interferons have been certified as the treatment for some diseases by FDA; some of these diseases are hepatitis 'B' and 'C', genital organ warts (Created by Papilloma), leukemia, blood cancer, flesh or connective tissue, multiple scleroma return or its and remitting and chronic granulomatosognor (13-15).

At present time interferons are used in following clinical tests:
Throat warts, Aids infection, chronic leukemia, colon tumors, kidney tumors, bladder cancer, melanoma, basic cell carcinoma and leishmaniasis. Of course, if the rates created by ozone become safe, interferons in FDA tests may not be fruitful in those conditions and be poisonous (16).

3 – Ozone produces TNF. TNF is produced in the body during a tumor growth; when the tumor is bigger, the tumor necrosis production increases, too. When a tumor is under metastatic process, the cells are separated and transferred to other parts of the body by blood and lymph; this process make the tumor choose another place in the body to stay; these cancerous cells have less chance for growth in order to resist against main tumor due to TNF production; when tumor is removed by surgery TNF rate decreases considerably and then new tumors grow from the tissue which seems sound (12).

4 – Ozone stimulate IL2 secretion. Interleulcin 2 is one of the bases of the body immunity system secreted by T-helper. In a process known as self-motivation IL2 joins a receiver on T-helper and produces more IL2; its main function is to stimulate lymphocytes and creates difference, multiplies and makes evident more cells such as T-helper, suppressors cytotoxicts, T-, T-delayeds, T-memory (13).

5 – Ozone destroys bacteria by the least focus on them. The metabolic effectiveness of most bacteria is averagely 1/17 of our metabolic efficiency; that is why mostly they cannot produce consumable antioxidant enzymes such as catalase; it is noteworthy that a little types of bacteria may live in a place with two percent ozone (15).

6 – Ozone may be used against any type of fungus and destroys sportsmen's leg contamination, moulds, white mould and yeasts (17).

7 – Ozone is one of the best antiviruses. As we said before ozone follows directly the viral particles. The most sensible part of the virus to oxidation is its multiplication system showing how viruses enter into the cell. If this structure becomes inactive, virus disappears. The cells contaminated before are naturally weak to ozone (19).

8 – Ozone is considered as an anti-neoplastic element namely it prevents new tissue growth because rapid cells' division disavows their responsibility regarding production of the enzymes necessary to protect themselves against ozone in a way that cancerous cells are decomposed rapidly, but such division marvelously stops beside ozone (20).

9 – Ozone oxides arterial plaques and neutralizes arteries' hardening namely ozone cleans narrow and even thick veins and this process facilitates oxidation of tissue in insufficient organs (21).

10 – Ozone increases red cells' flexibility and changeability. When we see a red cell under microscope it is like a disc; these discs stretch to become like an umbrella or oval where the capillaries collect oxygen (Lungs) and liberate it (Tissue); this process helps them to pass through small veins and ozone exchange becomes more efficiently (21, 22).

11 – This function accelerates citric acid cycle; this process is known as Kerp or TCA cycle which is an important step in glycolysis carbohydrate for energy. This process appears in mitochondria of the cell; so finally more reserved energy is converted in glucose (18).

12 – Ozone makes antioxidant enzyme system more efficient. The cells' reaction includes the increase of its protective enzymes production in proportion to useful oxidative pressure (22).

13 – Ozone decomposes petrochemical materials which may create some disorders for body's immunity system and may create allergy or worsen it in a way that these are defining factors in human's health (21).
The factors leading to chronic wounds:

**Diabetes:**

Diabetes is one of the most frequent causes of neuropathy in the organs; it is the cause of 2/3 of non-traumatic amputations due to diabetic leg. Generally it is possible to categorize neuropathies in three groups: sensory, motor and autonomic; the sensory neuropathy is the most frequent one in the patients suffering from diabetes; it makes the organs ready for ulcers due to disorder in sensory organs, destroying warm, pain and pressure sense (16). The studies indicate that these people are exposed to lower organs ulcers 15 times more than normal ones; also the tissues become more susceptible to such ulcers due to disorder in the arterial blood circulation because of vein artherosclerosis, stasis and in sufficiency in the patients; usually the ulcers begin from the leg exposed to pressure and alternative little traumas. Motor neuropathy deforms organ by creating disequilibrium between extensor and flexor muscles; such deformity and changed forms lead to weighting in the organ. The natural place to bear the maximum pressure is the sole of the foot, but it changes to the tip of the foot and base of the toes after deformity and put these points under continuous pressure and trauma. On one hand, this disequilibrium between flexors and extensors changes the position of protective pads of the sole of the foot so the joints above it become sensible to hurt. Autonomous neuropathy appearance has some role in the ulcer creation (19, 21) in a way that it disorders mechanisms related to sweating led to dry foot so makes it susceptible to ulcer creation; such dryness may create fissures creating susceptibility to ulcer creation and development (22).

**Vascular diseases:**

Essential mechanism creating arterial wounds are mostly due to lack of blood in final tissues. These wounds are often dry and painful and seem necrotic. Disorder in blood circulation renders these wounds' improvement difficult (23).

Pulmonary wounds appear due to blood stasis in the veins. Contrary to arterial ulcers whose base is pale the base of these ulcers is erythematous and wet. The edema due to excessive secretion of interstitial tissue, red cells' entrance into the interstitial tissue space, hemosiderin concentration and then hyperpigmentation and finally skin ulcer are of the changes appear after blood stasis in the veins and create some changes in the tissue and ulcers; these ulcers have usually irregular margin and may have loud smell. The vein ulcers usually appear on internal ankles (24).

**Ulcers due to pressure:**

As the title of the ulcer indicates the chronic and long ulcer on a defined place of body is the main cause of its creation; these ulcers usually appear where there is a subcutaneous osseous apophysis; sacrum is one of these places. Any disease leading to long rest in bed or some causes such as consciousness decrease for a long time (Coma) may create susceptibility to such ulcers; as mentioned before the ulcers due to pressure usually appear on osseous parts of the body because of low fat there and their weakness to protect skin; heels and hips are of the most frequent parts of this type of ulcers; base of vertebral column, shoulders, back, knee sides and nape. The ulcers may improve more satisfactorily by observing following points (24):

1 – Removing the pressure which is considered as the cause of ulcer.

2 – Treating the ulcer.

3 – Improving nutrition and creating the conditions appropriate to improve the ulcer better and more rapidly.
Ulcer due to osteomyelitis:

Osteomyelitis is a type of bone infection created as acute or chronic. Its transfer may be through blood or infectious neighbor tissues. Debridement or discharging dirt, infectious and necrosed tissues are necessary in acute osseous infections; if such tissues remain in the bone, it may lead to be chronic. Sequestrum (A piece of dead bone in a sheath of live bone, formed by necrosis and occurring esp. in cases of osteomyelitis. Also, a portion of skin separated by disease from the surrounding parts) sometimes appear after an acute osseous infection may be a proper place for the microbes' growth and multiplication. Blood circulation and antibiotics do not reach the necrosed tissues so the infectious factors are far from defensive and remedial mechanisms; such necrosed bone may become a place appropriate to chronic osseous infection. The most important diagnosis of chronic osseous infection is chronic dirt secretion leading to chronic ulcer; the dirt opens its way from bone towards skin and is secreted continuously. If the skin where there is the infection is healed, the dirt path is closed; in such cases the dirt is concentrated under skin and is expelled again from the same place after a while. Sometimes the path to expel the dirt is closed by the body; in such cases the diagnoses may appear as acute osteomyelitis with ague; however, when the dirt is expelled the diagnoses decreases and appear chronic. One of the most necessary treatments for these patients is to define the pathogenic microbes and antibiotics effective in treatment in a way that the latter be strong enough to fight the pathogenic factors, penetrate into the bone and may be prescribed for the patient for a long time. Surgery is necessary approximately in all cases. Then osteomyelitis improvement leads to improvement of the ulcer due to it (28).

Different types of ozone therapy:

Frequent methods of ozone therapy are as follows:

Major Auto - homoeopathy:

Sterile under vacuum bottles are used with anticoagulant in this method; this bottle is filled with patient's 50-250 ml vein blood; immediately the blood is mixed with ozone whose dose depends on the type of the disease and its progress and then is injected into his (her) vein (23).

Minor Auto - homoeopathy:

The method is used to create stimulation when the immunity system is weak. In this simple and easy method 5 – 10 ml vein blood with defined dose of ozone is injected into the patient's muscle (16).

Subcutaneous, intracutaneous, intramuscular and intra-articular injections:

A mixture of gaseous ozone – oxygen is used in all above cases. Ozone use has anti-inflammatory and pain killer effects.

Rectal enema is done with Janet syringe and special polychlorovinyl while the patient lies down on left side and bends his (her) knees. The intestine is prepared one hour before the enema. The rectal enema is done with ozone – oxygen mixture 10 – 60 ml per litre with amount of 150 – 1,000 ml depending on the pathologic step, its process and disease step (18).

Plastic bag containing ozone:

This method is most effective on dirt ulcers, bedsore and ulcers due to burning. Lower organs should be washed with water or saline and put into impermeable plastic bag before the operation. The air is discharged from the bag and filled with the gaseous mixture until having additional pressure and left in this condition for 15 – 20 minutes. Ozone with high concentration (6 – 8 mg/l ) is used to the patients...
suffering from vascular diseases, if their skin is not involved. The wound should be covered with the gas mixed with saline or ozonized water in the cases with dirty wounds; in this method first the ozone concentration for disinfection is 5 – 6 mg per litre and decreases to 1 – 2 mg per litre in the step creating the tissue granulation (19, 21).

**Ozonized water:**

Five mg per litre ozone is mixed with water in a bottle. The ozonized water is used vastly to wash surgical wounds and in genecology; it is used as a drink in gastro-intestinal diseases such as esophagitis gastritis and wounds and in intestinal inflammation for enema processes, too. Also it is used as mouthwash for oral diseases to disinfect the mouth in stomatitis and dental ulcers. One of the important points to use it is its usage for 30 minutes from the preparation (24).

**Ozonized vegetable oil:**

Antimicrobial activity of ozonized oil is several hundred more than ozonized saline. Pure vegetable oil is used to ozonation (Sunflower, olive, maize, etc.). Ozone and vegetable oil are mixed with different concentrations and times. Ozonized oil should be kept far from direct sunshine; hence, it is kept in dark bottles; if the maintenance is good, it may be kept in room temperature for four months and if it is kept in refrigerator, it may be used for two years. The dose is one tea spoon two to four times daily 20 minutes before meal and it may be increased to one soup spoon (21).

Actual methods to heal chronic wounds:

Usually three points are important in healing chronic wounds (28):

1 – Debridement and cleaning the ulcer dirt.

2 – Controlling microbial activity in related place.

3 – Covering the place well.

The first step in healing chronic ulcers is to clean the dirt and tissues without blood circulation; this process plays an important role in decreasing microbial activity and accelerating ulcer improvement. There are different methods to clean and for debridement and none of them is proved as a better one. Although none of debridement methods is better than others, generally debridement is done rapidly, effectively and cheaply by sharp devices and cutter; efficiency of this method is used especially to the ulcers due to pressure, vascular insufficiency and diabetes. The blood circulation should be sufficient in the tissue for the arterial ulcers and then the debridement is done (21).

Next step is to control chronic ulcers and microbes namely controlling bacterial activity and number. Tissue biopsy may be useful to examine the rate of the bacteria of an ulcer. Also swap may be used to sample the ulcer. The rate of the bacteria in the ulcer is 101 – 105 colonies per gram in tissue or any number of streptococcus beta hemolytic in the ulcer which are considered as harmful cases and encounter problems during ulcer healing. The most frequent pathogenic factor in the chronic staphylococcus aureus ulcers and resist against methicillin in about 20 – 50 percent cases. Local antibiotics may be used to decrease the bacteria in the ulcer; systemic antibiotics usage has indication in some cases indicating some systemic infections, cellulite or acute infection on the ulcer. Osteomyelitis may be with prescribing systemic antibiotics and surgical cases (31).

The third step controlling chronic ulcers is to cover it well. Humid balance in the ulcer and pain control are the measures to select the covering for the ulcer. Besides, this covering should be used easily and
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cover should be monotone for the ulcer and surrounding tissues and you should try to prevent the ulcer edges stretching. Many years ago it was imagined that the ulcer dryness may accelerate its healing, but now we know humidity may accelerate it. On the other hand, it should be noted that humidity and excessive secretion may have inverse result and macerate surrounding tissues. Ulcer humidity increases through hydrocolloid and hydrogel coverings and films while the foams, hydrofibers and dressing contain calcium alginate decreasing ulcer humidity. Generally hydrogens facilitate debridement; foams accelerate granulation and hydrocolloids are useful in epilation; however, saline or paraffin-gauze have been acceptable in recent studies (29, 30).

Mechanisms healing ulcer by ozone:

Ulcer healing is a complicated process including different steps such as hemorrhage, blood coagulation, inflammation, cell multiplication, granulation formation, reepithelialization and tissue rearrangement.

Ozone beside above mentioned treatments for chronic ulcers may be effective in healing ulcer. Until now different mechanisms have been proposed in relation to the effect of ozone in healing ulcer such as following cases (30 – 32):

**Increasing cellular healing processes:**

Local treatment by ozone activates kernel factor 'KB'. Many inflammatory molecules and growth factors such as TNFα, TGFβ, ICAM and VCAM begin to function after activation of this inflammatory factor. Inflammatory processes increase and cellular healing and multiplication occur when these molecules free in the ulcer place and then the ulcer healing is accelerated (44); specially TGFβ increase plays a vital role in rearranging tissue and then is necessary to heal the tissue. Different studies indicated that the endogenous growth factors are necessary for ulcer healing process; some of these factors are as follows: growth factor derived from PDGF plaquette, TGF-β and VEGF. These factors are resulted from secretion from fibroblasts, keratinocytes and macrophages and play some role in some processes such as granulated tissue formation, capillaries formation and collagen synthesis based on what indicated in previous studies.

It seems ozone therapy may free much of PDGF and TGFβ from the plaquettes and also another study examined ozone effect on healing ulcer indicated ozone therapy increased PDGF, TGF-β and VEGF in the ulcer place and then accelerated the ulcer healing; also ozone has been considered as a factor increasing PCNA and K10; the second molecule is keratin secreted from keratinocyte cells; these findings may indicate that ozone may be effective in multiplication process and keratinocytes distinction. Besides, it seems hyperbaric oxygen therapy may accelerate granulation tissue formation by increasing oxygen concentration on the ulcer. Oxygen in ischemic chronic ulcers induces VEGF accelerating ulcer closing and local treatment by ozone may increase local oxygen and then accelerate granulation tissue formation.

**Vascular function healing and blood circulation increase:**

Ozone therapy effect has been examined since 90 years ago and until now different studies have been done on the patients suffering from vascular artherosclerosis indicating better blood circulation in the subjects after ozone therapy. The tissue damage in vascular artherosclerotic diseases occur after blood circulation decrease in the tissue; besides, after tissue damage or infection creation the tissue healing encounters disorder due to blood circulation decrease. Different studies indicated ozone therapy increases glycolysis in red cells. One of the mechanisms involved in this process relates to the activity of glucose 1 phosphate dehydrogenase (28). The increase of this enzyme in red cell would increase glycolysis and antioxidant potential. It is noteworthy the comparison of ozone therapy effect with oxygen therapy in this field in a way that it seems ozone therapy is more efficient than oxygen therapy (21); increased adenosinediphosphate and 2 and 9 diphosphoglyglycerate are of other effects of glycolysis increase and increase of both of them in red cell lead to free more oxygen from hemoglobin and more ozone therapy
increases blood circulation in the tissue (22); also it increases local CO and NO and then vascular diastole occurs so blood circulation improves in the tissue (12).

The effect in healing ulcer infection:

Both inherent and acquired immunity play some role in cleaning the ulcer place from pathogens and in healing the ulcer, too; however, inability of the immunity system to clean the pathogens leads to chronic infection and then the growth factors have not the potential to induce cellular multiplication and heal the tissue and finally the ulcer closing encounter some disorders. One of the most important factors of chronic ulcers is the infection remaining in the ulcer place. Nowadays ozone is known as an efficient material to remove bacteria, viruses and fungi. Liquid ozone therapy removes Pyunkrutyk remains from the ulcer surface and accelerates the infection healing. It goes without saying that the accelerated infection healing accelerates the ulcer healing and the time necessary to heal the ulcer in the patients decreases (19). Some studies concerning ozone it is known as a material to induce immunity system and if it is added to plasma, it creates no poisoning and increases cytokines rate of the immunity system such as interferon gama and interleukin 6, 2 and 8. On the other hand, NFkB activation by ozone may lead to activate other immunity cells. By virtue of the role played by immunity system in healing and closing the ulcers it seems ozone therapy may be effective in healing ulcer (6, 17-21).

Conclusion

Ozone increases white cells in the blood and these cells protect body against viruses, bacteria, fungi and cancer and considering white cells may not activate and lose their potential to destroy attackers and even cannot resist against sound and normal cells without oxygen (23) ozone use in long term increases oxygen in blood so allergies decrease (32). Generally it seems ozone therapy by cellular multiplication processes induction and tissue healing, more oxygen in tissue blood and helping to clean the ulcer lead to accelerate ulcer healing. Nowadays the mechanisms to accelerate ulcer healing by ozone have been described and this material is used to help the ulcer healing in different countries throughout the world specially in Europe and South America. It seems by virtue of the findings from the effect of ozone on healing ulcer and previous studies (12, 21, 32) great clinical tests by proper designs may be an important step in healing chronic ulcers.

References

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